SURFACE MODIFICATION FOR BIOCOMPATIBILITY

Contract No. NS 5-2322

Quarterly Progress Report #2

July 31, 1995

The University of Michigan

David C. Martin and K. Sue O'Shea

Quarterly Progress to:	National Institute of Health
Contract Monitor:	William Heetderks, Ph.D.
Research Contract	"Surface Modification for Biocompatibility"
Contract No.	NS 5-2322
Principal Investigators:	David C. Martin and K. Sue O'Shea
Date:	July 31, 1995

Overview

This report is a summary of our activity in the second quarter of 1995. We continued our efforts to deposit and characterize microstucturally controlled bioactive protein polymer films on solid surfaces. We have also obtained data on the *in vivo* performance of these materials when implanted into the Central Nervous System (CNS). This report provides an overview of the major results to date and discusses our plans for the future. We have been working to evaluate (1) protein polymer film deposition and morphology, (2) bioactivity of protein polymer films *in vitro*, and (3) bioactivity and stability of protein polymer films *in vivo*. We also describe our efforts to discuss our work in (4) external communications with the scientific community.

1. Protein Film Deposition and Morphology

Progress:

We have completed a correlative study of protein polymer film deposition on solid silicon substrates using quantitative optical microscopy with digital image analysis and atomic force microscopy (AFM). From this information, we now know how to manipulate and control the film thickness, roughness, and microstructure as a function of protein solution concentration and applied electric field. At low concentrations, the film morphology consists of flattened droplets with an average diameter of 0.5 to 3.0 microns and average height of 50 to 500 nm.

Comparisons of the reflected light scattering from the protein films with the AFM data shows that the amount of light scattered from the film first increases, and then decreases as the thickness increases. This is evidently associated with a transition from a discontinuous to continuous film with increasing thickness. This has important implications for the expected electrical transport through the film. It is anticipated that a continuous film will be disadvantageous for maintaining efficient electrical communication between the implanted device and nervous tissue. Hence, we hypothesize that there may be an optimum thickness and morphology in which the amount and distribution of protein present at the surface is high enough to promote cellular ahesion, yet not too high to restrict the communication between the living tissue and the electronically active probe surface.

Plans:

Discussions with Prof. David Anderson in EECS have indicated that a useful means to examine the behavior of the probes with polymer coated surfaces is to explore the measured impedence as a function of signal frequency. Experiments on the uncoated

probes indicate a frequency-dependent impedence response over the 100-10000 Hz range. The impedence decreases systematically as the frequency increases in this frequency range.

We anticipate that experiments to characterize the probe performance as a function of frequency with different coatings will help to reveal the possible role of the protein polymer in restricting electronic transport from the probe to the tissue. We hypothesize that there may be characteristic features in the spectral response that can be used to reveal more detail about the mechanisms of probe performance, and may also be useful for the quality control during probe manufacturing.

2. Bioactivity of Protein Polymer Films *in vitro*

Additional studies to reveal the biological response of the protein films described in our first progress report are continuing to provide information about cellular adhesion as a function of protein coverage. These studies involve quantitative evalution of cell adhesion and spreading on protein polymer coated surfaces.

Plans:

PC12 (pheochromocytoma) cells are derived from the adrenal medulla, and differentiate into a relatively pure population of neurons in response to nerve growth factor (50 ng/ml). We are beginning experiments designed to test whether growth factors suspended in polymer coatings can be delivered to target neurons. A variety of concentrations of NGF are being suspended in protein polymers and used to coat coverslips on which target PC12 cells will be grown for periods of 1-5 days in vitro. These experiments will determine whether the growth factor will remain active and available to cells after processing, and will be important for future studies designed to attract and maintain neurons at the probe surface, and potentially to reduce mitotic activity of glial cells, which effectively isolate the probe surface from neurite contact.

3. Bioactivity of Protein Polymer Films *in vivo*

Progress:

Discussions with David Anderson of the Solid-state Electronics Laboratory and Josef Miller of the the Kresge Hearing Research Institute revealed their preference for using the Guinea Pig as a model system for examining the influence of *in vivo* response to coated substrates. Polypropylene suture (~50 micron diameter) was coated with the following materials and implanted in the Guinea Pig CNS:

1. no coating (control)
2. SLPF coated
3. SLPL coated
4. SLPF/Schwann cells
5. SLPF coated and exposed to CSF
6. SELP coated

The implants will be in place for periods of 3 and 12 weeks. The brains were removed, blocked, and evaluated by light and electron microscopy for information about tissue response, adherence of the Schwann cell layer, and stability of the protein film. The response of the tissue near coated micromachined probes will also be evaluated using laser confocal microscopy.

The first set of animals (3 weeks residence time) have now been evaluated histologically. The sample preparation involves embedding the tissue in poly(methyl methacrylate), sectioning with a stainless steel knife, and examination by optical microscopy. This effort has been conducted in collaboration with Rick Altschuler and coworkers of the Kresge Hearing Research Institute. Our results indicate that there was no marked inflammation near any of the probes after three weeks. A copy of an optical micrograph of a cross-section is enclosed. Note that the shape of the cells within 50 microns or so of the suture are somewhat distorted, while the morphology of the CNS further from the suture is similar to that of the pristine tissue. Further analysis of this encouraging data is underway.

Plans:

An additional set of three animals are being run with identical conditions to confirm and corroborate the results from this first study, and to explore the behavior of silicon probes coated with similar materials.

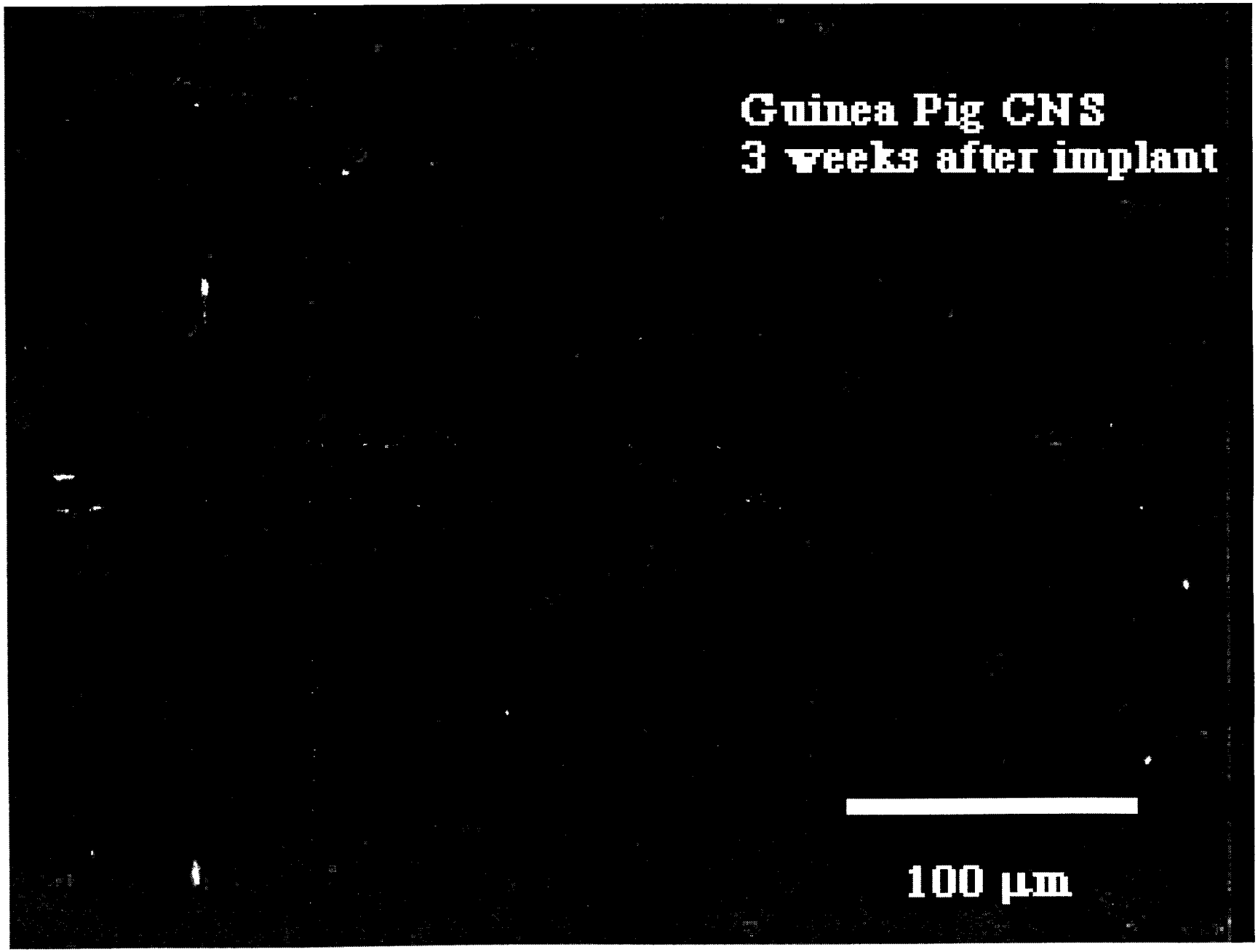
4. External Communications

A poster titled "Deposition and Chacterization of Biofunctional Polypeptides on Surfaces", by Chris Buchko, Ken Kozloff, J. Philip Anderson, Atisa Sioshansi, K. Sue O' Shea, and David C. Martin was presented at a meeting of the American Vacuum Society in Ann Arbor, MI on May 18, 1995.

An abstract titled "Electric Field Mediated Deposition of Bioactive Polypeptides on Neural Prosthetic Devices", by Chris Buchko, Ken Kozloff, Atisa Sioshansi, K. Sue O'Shea, and David C. Martin was submitted and accepted for presentation at the fall 1995 Materials Research Society meeting in Boston, MA. A copy of this abstract is enclosed.

An abstract titled "Glial and Neuronal Cell Response to Patterned Substrates Coated with Silk Polymers Containing Elastin, Fibronectin, or Laminin Cell Binding Motifs", by K. Sue O'Shea, Atisa Sioshansi, Chris Buchko, Joe Cappello, and David C. Martin was submitted and accepted for presentation at the the Society for Neuroscience meeting. This work was selected for a press release.

We are also currently working on an invited review paper on *Protein Polymer Processing and Characterization*, by David C. Martin, J. Philip Anderson, Chris Buchko, and Tao Jiang, which is scheduled to appear in a special volume on *Protein Polymer Materials*, edited by Kevin McGrath and David Kaplan of the U. S. Army Natick RD&E Center.


Guinea Pig CNS
3 weeks after implant
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ELECTRIC FIELD MEDIATED DEPOSITION OF BIOACTIVE POLYPEPTIDES ON NEURAL PROSTHETIC DEVICES

Christopher, J. Buchko*, Kenneth M. Kozloff*, Atisa Sioshansi*, K. Sue O'Shea, David C. Martin*†; *Materials Science and Engineering, **Department of Cell Biology, †Macromolecular Science and Engineering Center, University of Michigan, Ann Arbor, Michigan 48109.**

We have developed processing schemes for depositing three-dimensionally tailored layers of protein polymers on a variety of substrates. These polypeptides are genetically engineered to contain not only selected cell binding domains from naturally occurring extracellular matrix proteins such as fibronectin and laminin, but also silk-like sequences to provide structural integrity. One of our goals is to create stable, biocompatible coatings on silicon devices for implantation in the Central Nervous System. The ideal coating is one that encourages tissue to accept the neural prosthetic device, facilitates neurite outgrowth to the recording/stimulating sites, and prevents the migration of the device for long term stability. Our research has identified several candidate coatings whose morphology lies in the biologically significant 0.1 to 100 micron length scale.

Using electric field mediated deposition, we are able to process these polypeptides into biologically responsive films and coatings. Quantitative analysis of the structural-evolution of the coating enables us to fine-tune its morphology by varying parameters such as field strength and geometry or solution concentration. The interaction of the coated substrates with neurons and glial cells and device performance is examined *in vivo* and *in vitro*. Data collected from light optical microscopy, atomic force microscopy, transmission electron microscopy and scanning electron microscopy will be presented to give detailed insight into the nature of these coatings and their resulting properties.

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GLIAL AND NEURONAL CELL RESPONSE TO PATTERNED SUBSTRATES COATED WITH SILK POLYMERS CONTAINING ELASTIN, FIBRONECTIN, OR LAMININ CELL BINDING MOTIFS

K.S. O'Shea*[1], A. Sioshansi[2], C. Buchko[2], J. Cappello[3], and D.C. Martin[2], Depts. of Anatomy & Cell Biology[1], Materials Science & Engineering[2], Univ. of Michigan, Ann Arbor, MI, and Protein Polymer Technologies[3], San Diego, CA

The extracellular matrix (ECM) is important in both the development and response to injury of the nervous system. In the current investigation, we have examined glial and neuronal cell behavior on substrates coated with protein polymers produced in *E. coli* engineered to produce repeating silk like domains (GAGAGS) combined with ECM - cell binding motifs, e.g., the RGD sequence from fibronectin (SLPF), IKVAV from the laminin alpha chain (SLPL), or an elastin repeat (VPGVG; SELP). Neuro-2A, or neonatal Schwann cells were added (5×10^5 cells/ml) to coverslips coated with SLPF, SELP or SLPF (1 ug/ml to 1 mg/ml). On even very low concentrations of SLPF, both glial cells and neurons attached and there was significant glial cell spreading. On SLPL, neurons spread and extended neurites at all concentrations examined, while SELP coated substrates supported little adhesion of either cell type. These results suggest that bioengineered polymers reproduce cell-type specific behaviors typical of intact ECM molecules. Since recombinant protein polymers combine the processibility and stability of silk with biologically active moieties, they may form useful coatings for biocompatability.

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Key Words: (see instructions p. 4)

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2. Laminin
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